CORRECTED VERSION*

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/82, 15/62, C07K 14/00, A01H 5/00, C07K 14/08

A1

(11) International Publication Number: WO 00/36129

(43) Internati nal Publication Date: 22 June 2000 (22.06.00)

(21) International Application Number: PCT/SG98/00103

(22) International Filing Date: 11 December 1998 (11.12.98)

(71) Applicant *(for all designated States except US)*: INSTITUTE OF MOLECULAR AGROBIOLOGY [SG/SG]; 1 Research Link, Singapore 117604 (SG).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: FANG, Rong–Xiang [CN/CN]; Institute of Microbiology, Zhong Guan Cun, Beijing 100080 (CN). WU, Jun-Lin [CN/CN]; Institute of Microbiology, Zhong Guan Cun, Beijing 100080 (CN). CHEN, Xiao–Ying [CN/CN]; Institute of Microbiology, Zhong Guan Cun, Beijing 100080 (CN).

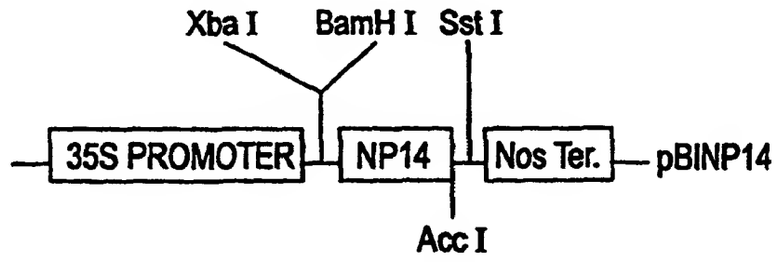
(74) Agent: ELLA CHEONG & G. MIRANDAH; P.O. Box 0931, Raffles City, Singapore 911732 (SG).

(81) Designated States: CN, JP, SG, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: ENHANCED PROTEIN PRODUCTION IN HIGHER PLANTS BY N–TERMINAL FUSION OF A UBIQUITIN OR A CUCUMBER MOSAIC VIRUS COAT PROTEIN PEPTIDE

(57) **Abstract**



Methods are disclosed for enhancing protein production. One method comprises preparing a vector by inserting a gene encoding ubiquitin in front of a gene encoding a protein of interest and inserting the vector into a cell. A fusion protein will be expressed which includes ubiquitin plus the protein of interest. Ubiquitin C–terminal hydrolases can cleave the fusion protein leaving the desired protein in its free state. This method causes enhanced production of the protein of interest as compared to performing the same method without the ubiquitin gene as part of the vector. A ubiquitin promoter is unnecessary to yield this enhanced production and is not used. A second method is very similar except that in place of a ubiquitin gene, a gene encoding fourteen amino acids of cucumber mosaic virus coat protein is inserted in front of the gene of interest. This results in expression of a fusion protein comprising the fourteen amino acid residues of the coat protein bonded to the protein of interest. The fusion protein is produced at a higher level than is the protein when the coat protein gene fragment is not present in the vector. In both methods the genes can be placed under the control of heterologous promoters such as a 35S promoter.

*(Referred to in PCT Gazette No. 41/2000, Section II)